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March 17, 2021	Final
R-21-0001 GENETIC CONSTRUCTION REPORT ANALYST: Khoa Nguyen, Ph.D. SUBMITTOR:	
INTRODUCTION  The submitter has engineered bacterial strains t	hey identify as,,,,,,,,,,,
,	of a single TERA case number (R-21-0001), as opposed to individua
	nism is "Bacilli strain engineered to affect nitrogen production"
(MCAN sec. 2.2). The confidential use is  The subject strains are intended fo to enhance nitrogen acquisition by plants. The genetic	r experimental environmental releases to evaluate their ability modifications were
The subject strains were developed using	
	strains are the result of and the
) containing an This was followed with transform	and the gene from , containing the genes of
interest flanked by	). These are recognized via the by the
, allowing for of the and are not maintained in	any of the final subject strains.
During the development of the subject strains, variou	s endogenous genes
. This resulte	
in the subject strains. The endogenous genes	/loci include ), and and
The functions of the disrupted genes	

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An overview schematic of how the subject strain was constructed is provided in Figure 1 and a detailed schematic is provided in the accompanying file (R-21-0001 GCR Schematic FINAL.pptx).

A summary of microbiological, genetic, and biochemical details related to the intergeneric genes in the subject strain is provided in Table 2 and corresponding details are provided in section II. The addition of intrageneric genes and the deletion of endogenous genes are addressed in sections II.D and II.E, respectively.



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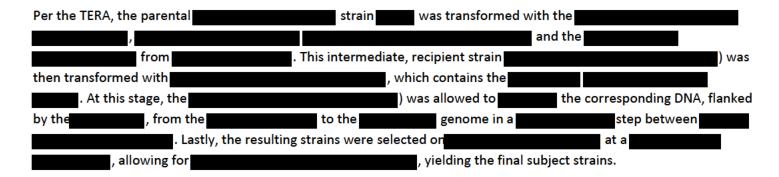
The subject strains are intended for experimental environmental releases to evaluate their ability to
enhance nitrogen acquisition by plants (specifically in corn plants; TERA sec 4.0). The genetic modifications were
(TERA sec 1.1).

### II. DESCRIPTION OF THE GENETIC MODIFICATIONS

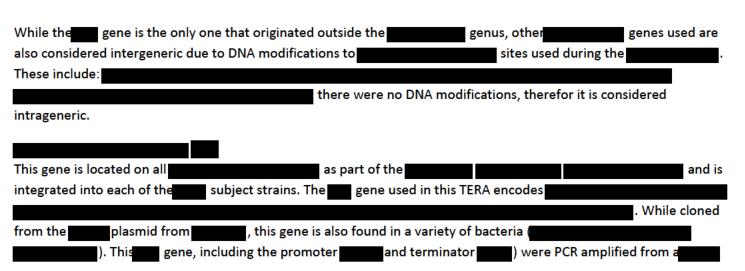
### A. The Parental and recipient strains

The parental strain is de	scribed as		
			This parental strain was then
transformed with a	,	carrying the	gene involved in
			This intermediate strain is
considered the recipien	t strain for a	ll eight subject strains. Since the submit	ter did not name this recipient strain, it will
be referred to as	for th	is assessment.	

#### **B.** Overview



### C. Addition of intergeneric genes



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	ble 3) with prime well as	r tails that affi	xed the to create	a	TERA	Att. 2).
e <b>1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1</b>			by			
	thereby gen	erating			).	
the TERA, this	gene					
					·	
	Figure 2. Illustrat	ion of				
	although the follo tions made to the			S	, they are considered for	d intergeneric due
s is in subject	strains					
subject strain						
	,					

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), This is in subject strain	
This is in subject strain	
Present in subject strain This codes for a and	from, consisting of
The submitter hypothesized th	at

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		I
This is in subject strain		
This gene codes for a from	).	
This is in subject strain		
This gene codes for a		

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# D. Addition of Intrageneric genes:

This is in subject strain .	

E. Deletion/Disruption	n of Endogenous Genes		
Per the TERA, the			

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The predicted integration site/disrupted gene is shown in the table below for each subject strains, along with their known functions.



### F. Step-by-step modifications

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The remaining details are well and concisely described in the TERA (Sec 2.2.3). An overview of the subject strains and their genotypes is in Appendix 1 of this report. TERA Attachment 2 contains information on the plasmids used to construct the subject strains. Sequence data are also provided in TERA Attachments 4-6.

A summary schematic of the genetic construction of the subject strains is provided in Figure 1.

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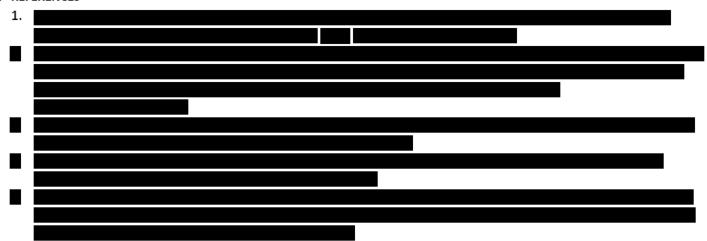
## **III. INSERTED SEQUENCE VERIFICATION**

strains.

from each subject strain	ı were	
(TERA sec 2.2.4). Sequencing was perfo	rmed using	which is an
adaptation of the	. prod	duced between reads
per sample. The maximum read length	was between	
The submitter confirmed that the	a	re intact and that the desired
containing the marker and	the genes of interest were inserted with	hout any deviation from the synthesized
sequence for all eight subject strains (T	ERA sec 2.2.3).	
On the issue of genetic stability of the i	nserted material, the submitter noted th	at all modifications were integrated
•	s of the genetic material has been observ	•
instances and many generations of cult	uring strains for research and developme	ent activities (TERA sec 2.2.5).
<u></u>		
	elements of the	used to engineer the subject
strains, the only one that remained in t		encoding a
from	providing	, providing
and	encoding the class	providing
like , were	part of the which do	es not remain in any of the final subject

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## IV. REFERENCES



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